Bat Foraging in Riparian Zones: Responses to Stream Quality, Insect Abundance and Season

By

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DEDICATION

The author would like to dedicate this thesis to his family, friends, especially Aileen Mooney, and advisors, especially Cynthia Walter, Ph.D., whom have all graciously supported and helped him immensely throughout his college career and thesis project.

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ABSTRACT

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Streams provide an important habitat for foraging among the nine species of insectivorous bats (family Vespertilionidae) found in Pennsylvania, particularly the common *Myotis lucifugus* (little brown bat). Many streams in Pennsylvania are negatively impacted by abandoned mine drainage (AMD), while an unknown number face further impending damage from Marcellus Shale natural gas drilling projects. The researchers attempted to determine the effects of stream quality on bat foraging. Stream benthic macroinvertebrate abundance was expected to be positively correlated with stream quality, and bat foraging was hypothesized to be higher in stream locations with higher water quality and associated high levels of insect abundance. Additionally, higher foraging was expected early in the season (April-May) compared to late in the season (September-October). To determine water quality, the researchers recorded the pH, water temperature, dissolved oxygen levels, conductivity and IBI of three forested streams in Westmoreland County, Pennsylvania with non-AMD-impacted upstream locations and AMD-impacted downstream locations. Habitat characteristics of the six stream sites were assessed using an EPA Rapid Bioassessment Protocol, and macroinvertebrate abundance was determined through D-net collection and subsequent identification and analysis. Bat foraging frequency was monitored by recording bat calls using a Binary Acoustic Technology AR125 125 kHz ultrasonic receiver for 20 minute intervals on suitable nights for a total of 17 recordings per site, and analyzed using Binary Acoustic Technology SCAN'R© analysis software. A three-Factor Analysis of Variance was used to determine if the stream location, stream quality (upstream vs. downstream), season, or their interactions affected bat foraging frequency, and stepwise multiple regression analyses were used to determine relationships between bat call frequency and habitat structure/quality, macroinvertebrate abundance, and weather measurements. Site, season, and their interaction all had a significant effect ($p < 0.05$) on bat call frequency. Regression analyses showed that habitat quality, stream quality, and macroinvertebrate abundance were good predictors of bat call frequency in spring, but instead air temperature was the strongest predictor in fall. In summary, the results suggest that stream quality, which affects macroinvertebrate abundance and subsequent insect biomass, affects bat foraging. Additionally, bats appeared to rely less on streams for foraging in fall than spring, and therefore stream quality measurements were not important in predicting bat foraging frequency later in the foraging season. Restoration and protection of streams will help provide an importance resource for bats in Pennsylvania and throughout the northeastern United States.

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INTRODUCTION

General Background

An assessment of bat foraging was conducted by determining the effect of stream quality and insect abundance on the frequency of riparian visits from foraging bats. Many bats, including all species present in Pennsylvania, are insectivorous, and many of them frequently forage for insects along bodies of water such as streams. The amount of foraging at any given time or place depends on a multitude of factors including time of night, time of year, physical attributes of the habitat, and numerous weather related factors. Additionally, foraging of insectivorous bats is related to insect abundance in an area. For stream and riparian foragers, water quality is an important factor for providing a healthy habitat for a large number of aquatic macroinvertebrates that mature into the bats' insect prey. Insectivorous bats use echolocation to navigate and forage for insects. In this assessment, bat frequency was monitored bioacoustically using an ultrasonic receiver, and macroinvertebrate abundance was determined using d-frame net samples. We compared paired sites upstream and downstream of coalmine drainage in three streams during two phases (April-May and September-October) to determine effects of water quality and season on bat foraging.

Of the more than eight-hundred species of the order Chiroptera, nine are found in Pennsylvania[6]. Each of these species are insectivorous, echolocating species of the family Vespertilionidae, and the most common of these bats, *Myotis lucifugus* (little brown bat), is found statewide across Pennsylvania. *M. lucifugus* is common in wooded areas and often forage along low-clutter environments[1], especially bodies of water[3,5,6,10]. In addition to *M. lucifugus*, there are three other less common species of the Myotis genus in Pennsylvania (*Myotis septentrionalis*, *Myotis leibii*, and *Myotis sodalis*) that share similar feeding and lifestyle habits as *M. lucifugus*. Other Pennsylvania bat species include *Lasiurus boreali*, *Lasiurus* *cinereus*, *Pipistrellus subflavus*, *Lasionycteris noctivagans*, and the common *Eptesicus fuscus* (big brown bat), which has also been found to have high abundance and frequency over streams[6,11].

In contrast to trapping, identifying and monitoring bats by recording echolocation calls provides a noninvasive, low labor cost method that can be applied over a wide area and that can provide detailed species-specific data[19].

Habitat and Foraging

On a smaller habitat scale, bat activity is often related to structural characteristics of the forest, proximity of riparian habitat, and characteristics of the bat species themselves (e.g., echolocation characteristics, wing morphology, and prey preference)[11]. The location of a bat at any instance can depend on a multitude of factors as well, such as time, temperature[7,8], humidity, precipitation[8,19], barometric pressure, wind speed[8,19], ambient light intensity[18], cloud cover, moonlight[8] and the availability and abundance of prey insects. Although cluttered forest areas often contain higher densities of insects, reduced clutter can result in relatively greater availability of insects because of the ease of maneuverability and movement while foraging in a more open area[11] and because of the reduced background noise that otherwise masks call echoes[17]. Streamside riparian zones are especially important for many insectivorous species because they provide favorable structural habitat by providing gaps that facilitate travel and foraging and by providing structural edges that are preferred by many species. Additionally, forested stream corridors allow bats to emerge earlier in the evening with a lower risk of predation by providing shelter from wind and suppressing light intensity to a degree. Furthermore, healthy streams provide an abundant source of prey insects for bats[14]. Nocturnal or crepuscular swarms of insects over water require less searching and are more conspicuous than prey in terrestrial habitats. Exploitation of aquatic swarms is particularly important for bats at high altitudes in Europe and North

America (e.g., *M. lucifugus*[3] and *E.* fuscus[23]) because these areas have habitats with numerous lakes and rivers[17].

Most bats species begin foraging around sunset[7]. Approximately 60-80% of the bats' daily food consumption occurs during the first two hours of foraging [3,18]. Anthony and Kunz found that this high consumption correlated with high insect availability[3]. Furthermore, Barclay showed that the high abundance of prey drops by almost an order of a magnitude a few hours after sunset[5].

Prey availability varies not only throughout a single night but also throughout the year due to temporal, seasonal and habitat-related effects on the mass of insects available[5]. Although overall resource densities are low in spring[18], Fukui et al. found that adult aquatic insect biomass was the highest in the spring and decreased gradually throughout the summer[14]. In contrast, terrestrial insects near riparian areas were lowest in the spring and gradually increased during the summer due to an increase in terrestrial plant productivity. Fukui et al. also reported that the location of bat foraging followed these trends in insect abundance throughout the year[14]. Results from multiple studies[3,18] showed that the seasonal feeding habits of temperate insectivorous bats change seasonally in response to not only insect abundance, but more importantly in response to changing energy demands during periods of mating, pregnancy, lactation, etc. These life history features in turn have evolved in response to the seasonal availability of resources[3,18]. Overall, food consumption in bats decreases in late summer and in autumn.

Streams and Water Quality

Due to varying tolerances of stream habitat features (e.g., substrate flow) and water quality (e.g., chemical characteristics, pH, oxygen levels, temperature) amongst aquatic macroinvertebrates, the presence of specific organisms can be used as an indication of stream quality. Streams affected by disturbances such as livestock grazing, incompletely treated sewage and suburban development have been found to sustain fewer than 20 taxa of macroinvertebrates compared to the 24-40 taxa of a typical pristine stream. Streams affected by abandoned mine drainage (AMD) show an even greater loss of diversity, often losing the ability to sustain macroinvertebrate life at all[22]. Lower macroinvertebrate diversities and densities within a stream result in lower adult aquatic insects around the stream, which reduces the availability of food for riparian foragers such insectivorous bats.

MATERIALS AND METHODS

Site Selection and Quality Analysis

Three streams impacted with AMD in Westmoreland County, PA were selected based on the known presence of bats and similarity in watershed composition of mixed agriculture and mature, second-growth deciduous forest. Upstream (U) and downstream (D) sites at Fourmile Run (F), Monastery Run (M), and Keystone (K) (i.e. Davis Run and McCune Run) were required to be as identical as possible to reduce the effects of confounding variables. Each second order stream site had a forested riparian habitat with modest canopy cover but with low levels of sub-canopy clutter (e.g., low hanging branches, bushes). Overall tree clutter of the surrounding riparian habitat was low to modest. In addition, stream and riparian landscape (e.g., stream width, number of riffles, level of stream debris and stream bank characteristics) was relatively similar for each site. Although similarity of these characteristics between sites at the three different streams was desired, similarity between each pair of upstream and downstream portions of each individual stream was imperative. However, upstream portions of each stream had high water quality and high insect abundance, whereas each downstream site had lower water quality due to AMD pollution and possessed lower insect numbers based on previous studies.

Each stream site was assessed using an EPA Rapid Bioassessment Protocol stream habitat assessment, which graded the sites on the following parameters: epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles (or bends), bank stability, vegetative protection of bank, and riparian vegetative zone width[4]. In addition, canopy height above the site was estimated. Canopy cover was determined by analyzing five canopy photos taken with a fish eyed lens along the length of stream at each site with Gap Light Analyzer, imaging software that extracts canopy structure and gap light transmission indices from true-color fisheye photographs[12]. Water quality at each site was determined by testing pH, water temperature, dissolved oxygen levels, and conductivity in spring with high water flow and again in late summer when water levels were the lowest.

Insect abundance was determined using an EPA Rapid Bioassessment Protocol for stream macroinvertebrates. Collection was performed by thoroughly kicking an approximately 1000 cm^2 area of riffle substrate immediately upstream of a D-frame net with 200-micron mesh for two minutes[22]; two samples were taken at two different riffles for each stream site. All macroinvertebrates in a sample were collected under 4x magnification with illumination and stored in 80% EtOH for later identification to the family level and separation of genera if possible. Macroinvertebrate abundance and pollution tolerance values[22] were analyzed to determine total taxa richness, EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa richness, Beck's index, Shannon diversity index, Hilsenhoff biotic index, and percent of sensitive individuals in order to calculate the index of biotic integrity (IBI) for each stream site[20].

Bat Call Monitoring

Bat frequencies were determined through Bioacoustics; bat calls were monitored using Binary Acoustic Technology LLC AR125 125 kHz ultrasonic receivers. Data collection occurred on nights when weather conditions were appropriate (zero or low wind speeds, minimal levels or absence of precipitation and temperatures above 10°C) and occurred shortly after the sun set until all six sites had been monitored. The AR125 recorder was set up on a tripod so that the recorder was focused between the water surface and 2 m above the water, which is the area that *M. lucifugus* has been found to forage frequently[3,5]. Bat calls were recorded onto a laptop for a 20 minute period using SPECT'R III (spectral analysis, digital tuning, and recording) software from Binary Acoustic Technology LLC. Weather measurements (ambient temperature, relative humidity, barometric pressure and wind speed) were recorded using a Kestrel 4500NV Pocket Weather Tracker during the call recording. Additionally, recordings of cloud cover using an ordinal scale ranging from 0-5 and moon phase from 0-100% occurred. Recording at each site was synchronized as closely as possible; a field assistant set up a second recorder and laptop and performed an identical procedure at the opposite stream location on the same stream upon which the first was set. Once 20 minutes of bat calls were recorded at the two sites of the first stream, the recording was stopped and the equipment was collected.

This monitoring process was repeated for both of the other streams, with as little time allowed to pass in transit between streams in order to take advantage of the period with greatest bat foraging activity, especially within the first two hours after sundown[3,8,18]. The order that the streams were sampled was randomized each night; however, both sites of a given stream were always sampled concurrently. Data collection occurred in two phases: nine nights between late April and May and eight nights from September through October.

Bat calls recorded throughout the data collection periods using the SPECT'R software were analyzed using Binary Acoustic Technology's SCAN'R (snapshot characterization and analysis) software to determine the number of calls that were recorded each night. On two occasions in the spring and five occasions in the fall, recordings for a site were invalid due to a temperature drop, sound interference or corrupted sound files. In these infrequent cases, counts were estimated by averaging the number of calls at the specific site on all other nights for that season.

Statistical Analysis

The effects of season (spring vs. fall), stream (FR, MR, or KS), and site (up- vs. downstream) on the frequency of bat calls were analyzed using a three-factor analysis of variance (ANOVA). Two-sample t-tests were used to compare bat call frequency between site pairs and between sites across seasons. Stepwise multiple regression models were used to determine the effect of macroinvertebarte abundance, habitat score, IBI, canopy cover, stream, time after sunset, air temperature, relative humidity, barrometric pressure, wind speed, cloud cover, observed moon phase, and actual moon phase (%) on the frequency of bat calls for both spring and fall. The variables were accepted into the models if they had a probability-of-F ≤ 0.05 and rejected if the probability-of-F was ≥ 0.10. The frequency of bat calls per 20 minutes was $\log_{10}(x)$ transformed to reduce heteroscedasticity. A *P*-value of 0.05 was defined as statistically significant for all tests. All statistical analyses were calculated using SPSS Statistics ver. 19 (IBM Corp., Somers, NY, USA). Data are presented as means ± S.E. unless otherwise stated.

RESULTS

In spring, FU and MU had approximately tenfold higher average abundance of bat calls than FD and MD respectively. In K sites, calls were consistently low and KD had about twice the number of calls than KU (Fig. 1).

In fall, only MU had a higher average number of bat calls recorded per 20 minute interval per night than its coinciding downstream site; FD and KD both had more bat calls than FU and KU respectively (Fig. 1). Overall, the sites had a higher average number of bat calls in spring (2057 ± 1010 calls) than in fall (561 ± 215 calls; Fig. 1). The three-factor ANOVA showed significant effect for season, $F(1, 90) = 8.38$, $p = 0.005$; site, $F(1, 90) = 6.45$, $p = 0.013$; the interaction effect of season and site, $F(1, 90) = 11.48$, $p = .001$; the interaction effect of stream and site, $F(2, 90) = 3.16$, $p = 0.047$; and the interaction effect of all three factors, $F(2, 90) = 3.54$, $p = 0.033$, on the frequency of bat calls. Stream ($F(2, 90) = 2.72$, $p = 0.071$) and the interaction effect of season and stream ($F(22,90) = 1.54$, $p = 0.220$) did not have a significant effect on the number of bat calls recorded.

The sites at keystone were found not to fit into the experimental design because KU had much worse habitat quality, although its water chemistry was slightly better than KD. A three factor ANOVA excluding the Keystone site data showed that season, $F(1, 60) = 7.40$, $p = 0.009$; site, $F(1, 60) = 7.80$, $p = 0.007$; and the interaction effect of season and site, $F(1, 60) = 12.35$, $p = .001$, still had a significant effect on the number of bat calls recorded, and that stream ($F(1, 60) = 0.02$, $p = 0.898$) and the interaction effect of season and stream ($F(1, 60) = 0.24$, $p = 0.625$) did not have a significant effect on the number of bat calls. However, the ANOVA showed that the interaction effect of stream and site ($F(1, 60) = 0.68$, $p = 0.415$) and the interaction effect of all three factors ($F(1, 60) = 0.11$, $p = 0.741$) no longer had a significant effect on the number of bat calls recorded.

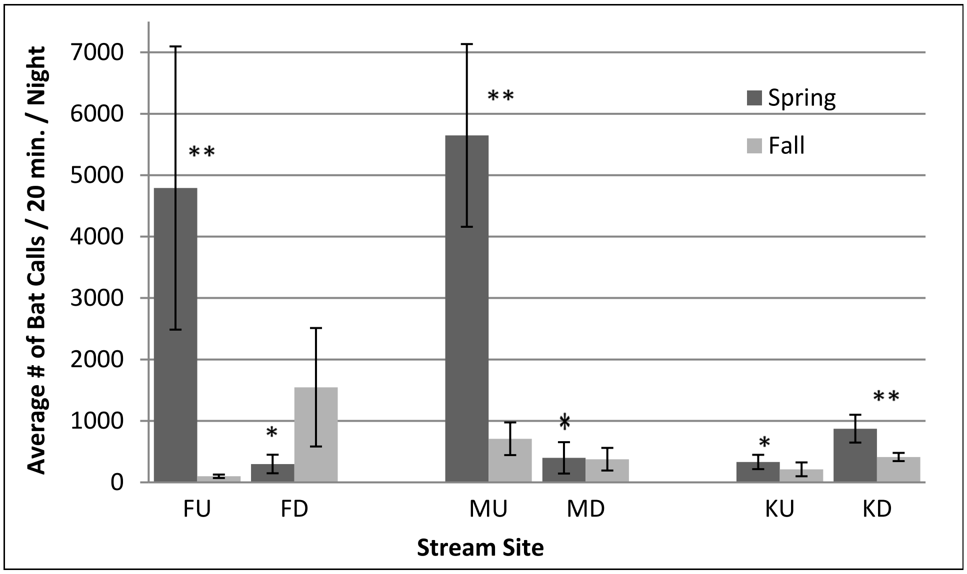

Figure 1. Seasonal comparisons of average number of bat calls recorded per 20 minute interval per night

Comparisons of the average number of bat calls recorded per 20 minute interval per night during spring and fall recording seasons. Key: FU/FD = Fourmile Run upstream & downstream, MU/MD = Monastery Run upstream & downstream, KU/KD = Keystone sites upstream & downstream (Davis Run and McCune Run respectively). Two-sample t-tests showed that there was a significant ($p < 0.05$) difference between all site pairs in spring, but not between any site pairs in fall. In spring, FU had more calls than FD, t(8) = -1.94, MU had more calls than MD, t(8) = -3.48, and KD had more calls than KU, t(12) = 2.13. There was also a significant difference in call frequency between seasons; FU (t(8) = 2.04), MU (t(8) = 3.28) and KD (t(9) = 1.97) each had more calls in spring than fall. **significance between seasons, *significance between site-pairs.

The abundance of macroinvertebrates caught at each upstream site was higher than the abundance caught at each downstream site of each stream for both seasons (Table 1). The average difference between paired-sites was approx. 200 ± 47 macroinvertebrates. The IBI score was higher for each upstream site than downstream site of each stream in both seasons except between KD and KU in spring (Tab. 1). The average IBI was consistently low for all sites between seasons; the average IBI in spring was 30.97 ± 3.78, and in fall it was 38.58 ± 7.34 out of a possible 100 (Tab. 1). The average difference in IBI between site pairs was 14.70 ± 4.90. The fall KU site had very high abundance and IBI due to an atypical appearance of a large number of approx. 250 young, low pollution-tolerance stonefly larvae of the family *Perlodidae* in one sample. The total habitat score was higher for FU and MU than FD and MD respectively, but was higher for KD than KU (Tab. 1). The average difference in scores between stream site pairs was approx. 32 ± 1.

The dissolved oxygen (DO) levels were higher in each of the upstream sites compared to each site's coinciding downstream site in spring and between Fourmile sites in fall, but were greater in downstream sites than upstream sites at both Monastery and Keystone in fall (Tab. 1). The average DO was greater in spring (9.79 ± 0.35 mg/l) than in fall (8.35 ± 0.56 mg/l). The conductivity of each downstream site was higher than the conductivity of each upstream site on each stream for both seasons. Overall, the average conductivity of the sites was higher in fall (818.83 ± 176.93 µS/cm) than in spring (503.75 ± 105.35 µS/cm; Tab. 1). Stream temperatures ranged between 17.2°C and 28.0°C in spring and between 15.0°C and 22.1°C in fall (Tab. 1). Additionally, the temperatures between sites of the same stream varied less than 1°C for each stream except for between sites at Monastery and Keystone in spring, which varied by 1.5°C and 4.1°C respectively. Stream pH remained relatively circumneutral among the sites in spring, but varied more in fall with concentrations of hydronium ions varying up to 2 orders of magnitude between sites and overall pH of the sites ranging from 5.5 at FD to 9.35 at MU (Tab. 1).

Table 1. Macroinvertebrate Abundance and Stream Quality Measurements of Stream Sites

Season	Stream / Site	Avg. # of Bat Calls / 20 min. / Night	Macro-invertebrate Abundance	IBI	Total Habitat Score	DO (mg/l)	Cond. (µS/cm)	Temp. (°C)	pH
Spring	FD	299 (± 151)	11	19.58	103	10.00	588	17.6	7.56
	FU	4791 (± 2305)	317	38.63	133	10.90	539	17.2	7.04
	MD	400 (± 255)	11	20.39	93	9.70	792	19.8	6.99
	MU	5647 (± 1487)	276	29.61	128	10.45	718	18.3	7.16
	KD	874 (± 26)	15	40.14	133	8.54	226	28.0	7.06
	KU	332 (± 116)	114	37.46	101	9.12	159	23.9	7.07
Fall	FD	1549 (± 964)	4	18.73	103	7.68	1146	21.6	5.50
	FU	100 (± 28)	192	43.90	133	9.18	1030	22.1	7.77
	MD	377 (± 184)	43	28.35	93	10.51	1194	20.8	8.74
	MU	711 (± 266)	75	30.03	128	8.35	1010	20.8	9.35
	KD	414 (± 67)	16	40.04	133	7.90	273	15.0	8.69
	KU	213 (± 113)	325	70.42	101	6.50	260	15.7	8.59

note: IBI = index of biotic integrity, DO = dissolved oxygen, Cond. = conductivity

FD had a canopy height of approx. 18m and an average canopy cover of 31.2%, while FU had a canopy height of approx. 25m and an average canopy cover of 24.0%. MD had a canopy height of approx. 16m and an average canopy cover of 18.3%; MU had a canopy height of approx. 20m and an average canopy cover of 22.4%. KD had a canopy height of approx. 20m and an average canopy cover of 14.8%; KU had a canopy height of approx. 20m and an average canopy cover of 14.8%.

The stepwise mutliple regression analysis created a five variable model for spring data with an adjusted R^2 of 0.510 and a standard error of the estimate of 0.540 ($F(5) = 12.04$, $p < 0.001$; Table 2). The significant variable predictors in the model were habitat score ($t = 3.75$, $p < 0.001$), macroinvertebrate abundance ($t = 3.10$, $p < 0.01$), cloud cover ($t = -2.37$, $p < 0.05$), air temperature ($t = 2.93$, $p < 0.01$), and IBI ($t = -2.12$, $p < 0.05$). The regression analysis created a three variable model for fall data with an adjusted R^2 of 0.357 and a standard error of the estimate of 0.651 ($F(3) = 8.15$, $p < 0.001$; Tab. 2). The significant variables predictors in the model were air temperature ($t = 4.04$, $p < 0.001$), macroinvertebrate abundance ($t = -2.64$, $p < 0.05$), and relative humidity ($t = 2.22$, $p < 0.05$).

In spring, the average air temperature was 17.8 ± 0.8°C ranging from 7.8°C - 23°C; the average relative humidity was 72.1 ± 1.6% ranging from 55.8% – 91.3%; the average barometric pressure was 29.02 ± 0.02 inHg ranging from 28.73 – 29.25 inHg; the average wind speed was 0.38 ± 0.09 mph ranging from 0 – 1.4 mph; the average cloud cover score was 1.1 ± 0.3 ranging from 0 - 5; and the average moon visibility percentage was 23 ± 6% ranging from 0% - 100%. In fall, the average air temperature was 17.3 ± 0.6°C ranging from 10.5°C – 22.5°C; the average relative humidity was 68.3 ± 1.4% ranging from 56.5% – 85.7%; the average barometric pressure was 28.86 ± 0.03 inHg ranging from 28.29 – 29.08 inHg; the average wind speed was 0.40 ± 0.07 mph ranging from 0 – 1.3 mph; the average cloud cover score was 1.1 ± 0.4 ranging from 0 - 5; and the average moon visibility percentage was 29 ± 7% ranging from 0% - 100%.

Table 2. stepwise multiple regression outputs for spring and fall seasons

Multiple Regression Models					Standardized Beta Values of Variable Components					
Season	df	R^2	Adjusted R^2	F	Habitat	Macro. Abundance	IBI	Air Temp.	Cloud Cover	Relative Humidity
Spring	5	0.556	0.510	12.039^{***}	0.551^{***}	0.375^{**}	-0.287^{*}	0.297^{**}	-0.230^{*}	-
Fall	3	0.357	0.313	8.152^{***}	-	-0.319^{*}	-	0.726^{***}	-	0.399^{*}

note: dependent variable: Log_{10} transformation of bat call abundance; *** $p < 0.001$, **$p < 0.01$, *$p < 0.05$.

All recordings were completed within the first three hours after sunset in spring with all but the last stream each night being recorded within the first two hours after sunset, and each recording was completed within the first two hours after sunset in fall.

DISCUSSION

Visual observations and call data support past studies demonstrating that insectivorous bats forage over streams[1,3,5,6,10,14,23]. The variation in bat call frequency was significantly due to site selection, season, and the interaction between season and site. In spring, sites with better water quality, better habitat and higher macroinvertebrate abundance (FU and MU) had more bat calls than downstream lower-quality sites (Fig. 1; Tab. 1). However, KD, which had a better habitat score than KU, had significantly more calls than KU even though KU had more macroinvertebrates and better water quality (Fig. 1; Tab.1). In fall, there was no significant difference in calls between site-pairs at all three streams. Overall, sites had more calls in spring than fall, especially sites that had more calls than their paired site in the spring (Fig. 1). Regression analyses showed that habitat quality, stream quality (IBI), and macroinvertebrate abundance were significant predictors of bat call frequency in spring, but instead air temperature was the strongest predictor in fall (Tab 2).

These results suggest that water quality and macroinvertebrate abundance (and therefore subsequent adult aquatic insect biomass) affects bat foraging frequency over streams, as demonstrated in past studies[5,14]. AMD-impacted streams had lower water quality (lower DO, higher conductivity, and lower IBI) and lower macroinvertebrate abundance than good-quality sites, which supports past studies[22] that found a correlation between AMD-impacted streams and lower macroinvertebrate abundance. Our results also suggest that bat foraging activity varies between the early foraging season and late in the foraging season. Our findings of higher call frequency at stream sites in spring compare to results found by Fukui et al. for bats in Japan[14]. They demonstrated that bat foraging positively correlated with the abundance of aquatic insects available in the spring, and that the bats relied less on streams for food later in the foraging season as terrestrial insect abundance increased. Because bats rely less on streams in fall, water quality appears to no longer be an important predictor of bat foraging activity late in the season as our multiple regression analysis demonstrates. Instead, increasingly colder nights had the strongest effect on foraging activity in fall, which is most likely due to associated drops in insect abundance[3,8]. Seasonal differences in bat activity have also been shown to be related to changing energy demands of the bats throughout the season[3,18].

Macroinvertebrate abundance was a significant negative predictor of bat calls in the fall, which is not consistent with spring correlations of higher macroinvertebrate abundance, adult insect abundance, and bat foraging activity. However, macroinvertebrate abundance increases in the fall due to increases in leaf-litter input and periphyton biomass from higher insolation[14], and therefore, higher macroinvertebrate numbers can result independent of higher adult insect abundance or bat foraging activity at that time. Additionally, IBI as a negative predictor of bat activity in the spring contradicts the idea that better water quality is correlated with more bat activity. However, the IBI analysis relies heavily upon pollution tolerant species, regardless of the total abundance of macroinvertebrates present. Past studies suggest that bats are opportunists and take advantage of swarms of aquatic insects[3,14] regardless of pollution tolerances. In the majority of sites we caught a high number of high-tolerance macroinvertebrates (data not shown), and thus total macroinvertebrate numbers would probably be a better indicator of bat foraging activity. Furthermore, additional measures of stream chemistry are needed to better predict stream quality and insect abundance.

Future studies would benefit from more sampling nights and longer sampling times each night, as well as more replication across streams and bioregions. Additionally, species identification of calls would be useful in determining which bat species are frequently using streams for foraging. Finally, examining light intensity and adult insect abundance directly would further help explain trends and changes in bat foraging activity.

Streams in Pennsylvania are already negatively impacted by fertilizer runoff, sedimentation and AMD[22]. Further degradation as a result of illegal dumping, improper storage of polluted water, and erosion associated with exponential increases in Marcellus Shale drilling projects across the state also seems imminent. Additionally, bats, especially *M. lucifugus,* are already experiencing unprecedented mortality rates across the eastern U.S. from the fast-spreading white nose syndrome epidemic that has already spread to more than nine states and Canada and that is expected to diminish northeastern hibernating bat populations to as low as 1% of their current population in less than 20 years[13]. With this epidemic already causing alarming decreases in bat populations, it is imperative to restore and protect streams to help conserve bats in Pennsylvania and the eastern United States.

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APPENDIX A

Site Locations:

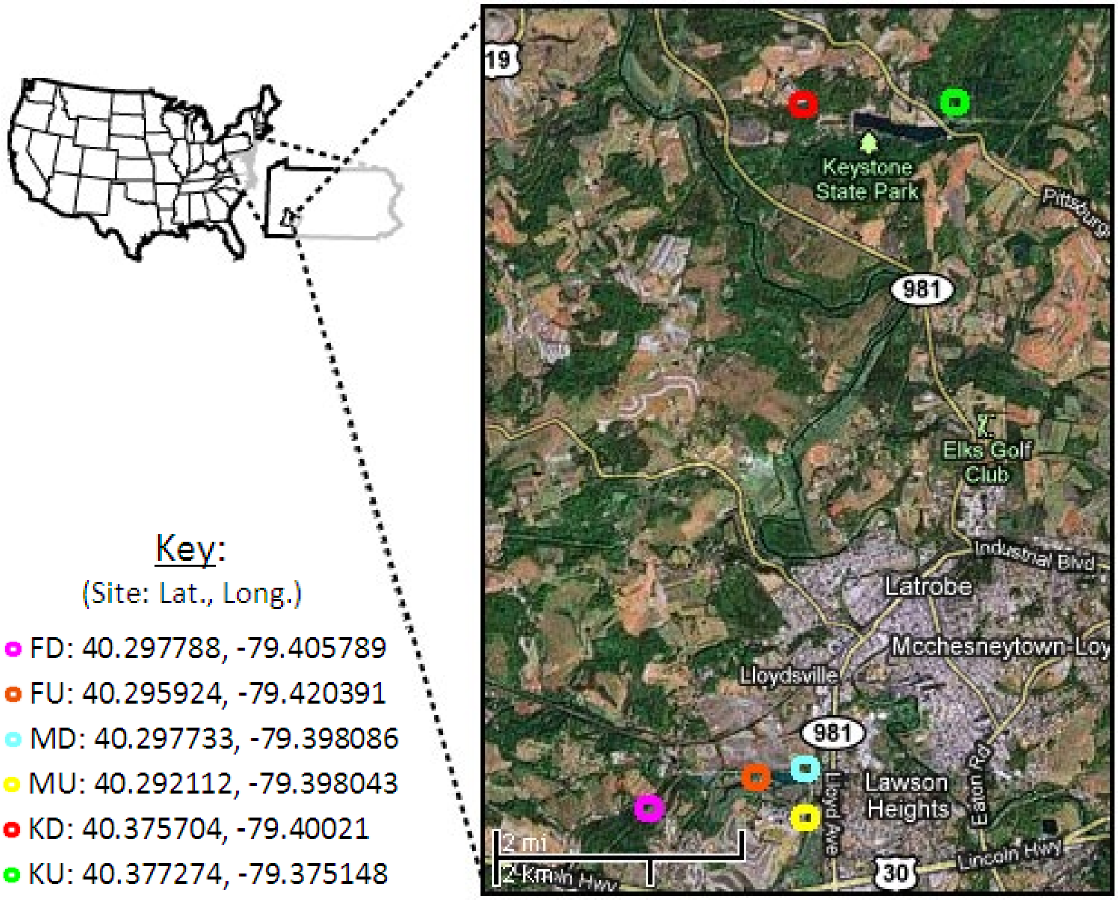
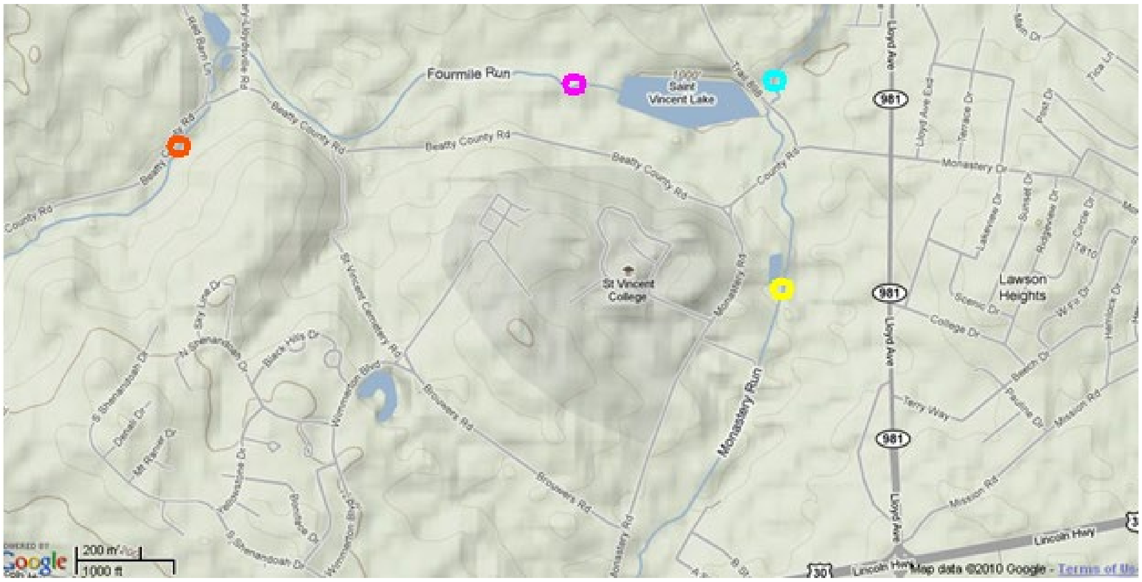


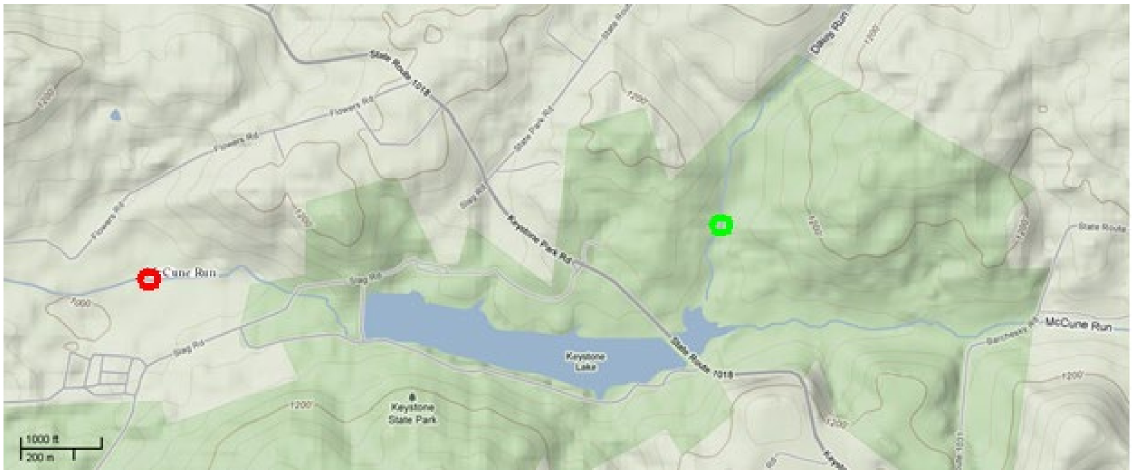
Fig. I. Site locations. Streams in Westmoreland County, PA: Fourmile Run, Monastery Run, and Davis/McCune Runs. The upstream site of each stream was within 2 km of a coinciding downstream site of the stream that received water contaminated by abandoned coal mines. Also, each stream was within approximately 2 km of a lake or pond.

Figure. II. Monastery Run and Fourmile Run:



- 🟠 FU: 40.295924, -79.420391; approximately 400 m upstream of confluence of Fourmile Run and tributary at Beatty Road
- 🟣 FD: 40.297788, -79.405789; approximately 1500 m downstream of confluence of Fourmile Run and tributary at Beatty Road
- 🟡 MU: 40.292112, -79.398043; approximately 800 m upstream of confluence of Monastery Run and Fourmile Run East of Saint Vincent Lake
- 🟢 MD: 40.297733, -79.398086; approximately 100 m downstream of confluence of Monastery Run and Fourmile Run East of Saint Vincent Lake

Figure III. Davis Run and McCune Run flowing in and out of Keystone Lake:



- 🟢 KU: 40.377274, -79.375148; approximately 300 m upstream of Keystone Lake
- 🔴 KD: 40.375704, -79.40021; approximately 1000 m downstream of Keystone Lake

APPENDIX B

EPA Rapid Bioassessment Protocol stream habitat assessment:

WATER QUALITY NETWORK HABITAT ASSESSMENT

Habitat Parameter	Category			
	Optimal	Suboptimal	Marginal	Poor
1. Instream Cover (Fish)	Greater than 50% mix of boulder, cobble, submerged logs, undercut banks, or other stable habitat.	30-50% mix of boulder, cobble, or other stable habitat; adequate habitat.	10-30% mix of boulder, cobble, or other stable habitat; habitat availability less than desirable.	Less than 10% mix of boulder, cobble, or other stable habitat; lack of habitat is obvious.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
2. Epifaunal Substrate	Well developed riffle and run, riffle is as wide as stream and length extends two times the width of stream; abundance of cobble.	Riffle is as wide as stream but length is less than two times width; abundance of cobble; boulders and gravel common.	Run area may be lacking; riffle not as wide as stream and its length is less than two times the stream width; gravel or large boulders and bedrock prevalent; some cobble present.	Riffles or run virtually nonexistent; large boulders and bedrock prevalent; cobble lacking.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
3. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
4. Velocity/Depth Regimes	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score lower than if missing other regimes).	Dominated by 1 velocity/depth regime (usually slow-deep).
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
5. Channel Alteration	No channelization or dredging present.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	New embankments present on both banks; and 40-80% of stream reach channelized and disrupted.	Banks shored gabion or cement; over 80% of the stream reach channelized and disrupted.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Total Side 1 ________				

Habitat Parameter	Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
6. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from coarse gravel; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, coarse sand on old and new bars; 30-50% of the bottom affected; sediment deposits at obstruction, constriction, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
7. Frequency of Riffles	Occurrence of riffles relatively frequent; distance between riffles divided by the width of the stream equals 5 to 7; variety of habitat.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is between ratio >25.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
8. Channel Flow Status	Water reaches base of both lower banks and minimal amount of channel substrate is exposed.	Water fills > 75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
9. Condition of Banks	Banks stable; no evidence of erosion or bank failure.	Moderately stable; infrequent, small areas of erosion mostly healed over.	Moderately unstable; up to 60% of banks in reach have areas of erosion.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; on side slopes, 60-100% of bank has erosional scars.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
10. Bank Vegetative Protection	More than 90% of the streambank surface covered by vegetation.	70-90% of the streambank surface covered by vegetation.	50-70% of the streambank surfaces covered by vegetation.	Less than 50% of the streambank surface covered by vegetation.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
11. Grazing or Other Disruptive Pressure	Vegetative disruption, through grazing or mowing, minimal or not evident; almost all plants allowed to grow naturally.	Disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	Disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Disruption of vegetation is very high; vegetation has been removed to 2 inches or less in average stubble height.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
12. Riparian Vegetative Zone Width	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE ________	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Total Side 2 ________				
Total Score ________				

APPENDIX C

Literature Review:

An assessment of bat foraging will be conducted by determining what effect stream quality and, by relation, insect abundance have on the frequency of riparian visits from foraging bats. Although there is a wide variety of bats with various feeding habits and that prefer a wide range of habitats, many bats, including all species present in Pennsylvania, are insectivorous and frequently forage for insects along bodies of water such as streams. The amount of foraging at any given time or place is dependent on a multitude of factors including time of night, time of year, physical attributes of the habitat, and numerous weather related factors. Additionally, foraging of insectivorous bats is related to insect abundance in an area. For stream and riparian foragers, the quality of the water is an important factor in order to provide a healthy habitat for a large number of aquatic macroinvertebrates that mature into the bats' insect prey. Insectivorous bats use echolocation to navigate and forage for insects. In this assessment, bat frequency will be monitored bioacoustically using an ultrasonic receiver, and insect abundance will be determined using sticky traps. The project will use a pair of sites upstream and downstream of coal mine drainage of three streams, and it will be carried out in two phases (April-June and then August-October) to determine the seasonal affect on bat foraging.

General Species Information:

Of the more than eight-hundred species of the order Chiroptera, nine species are found in Pennsylvania (Brack, 2009). Each of these species belongs to the family Vespertilionidae, also known as the evening bats or common bats, and they are all insectivorous. The most common of these bats, the little brown bat (*Myotis lucifugus*), is found statewide across Pennsylvania. *M. lucifugus* is, as its name suggests, a small bat (Length: 7.87 - 9.4 cm; wingspread: 21.8 – 26.7 cm; weight: 7-10 g) with fine and glossy brown fur. *M. lucifugus* hibernate in caves, mineshafts and tunnels from October or November until April or May, but emerge during the summer months to forage and bear and raise young. Males are solitary, while females gather to form summer nursery colonies; a single pup is usually born in June or July and is fully grown in four weeks. *M. lucifugus* are common in wooded areas and often forage along bodies of water (Fergus, 2010).

In addition to *M. lucifugus*, there are three other species of the Myotis genus in Pennsylvania that are similarly small and that share similar feeding and lifestyle habits as *M. lucifugus*. Northern long ear bats (*Myotis septentrionalis*) are much less common than little browns and more irregularly distributed across the state. They are similar in appearance as *M. lucifugus* and hibernate from November to March. The small-footed bat (*Myotis leibii*) is even less common and its rarity in Pennsylvania has caused it to be placed on the state's threatened species list. *M. leibii* is smaller than *M. lucifugus* and has smaller feet. Not much is known about this species other than it hibernates from November to March and that its lifestyle and habitat is probably similar to that of *M. lucifugus*. Indiana bats (*Myotis sodalis*) are similar in size as the little browns but have a slight pinkish cast to their brown fur. This species is on the federal endangered species list, and as a result is less common in Pennsylvania than other species; all members of the species travel to caves in Indiana, Illinois, Missouri, and Kentucky to hibernate (Fergus, 2010).

Big brown bats (*Eptesicus fuscus*) are also very common across Pennsylvania and can be found frequently in cities, countrysides, and wooded areas, especially deciduous forests. *E. fuscus* have darker brown fur and are larger than the small brown Myotis; they are between 10 and 12 cm long, have a wingspread of about 30-33 cm and weigh approximately 11-16 g. They are among the last bats to hibernate in the fall entering in October or December, and they emerge in March or April. Pups are born from May to July. Big browns learn to fly within a month and are weaned about two weeks later (Fergus, 2010).

Other bat species include the Red (*Lasiurus borealis*), Hoary (*Lasiurus cinereus*), Eastern Pipistrelle (*Pipistrellus subflavus*), and Silver-Haired (*Lasionycteris noctivagans*) bats. *L. borealis* is slightly smaller than *E. fuscus* and has a bright rusty coat and long, pointed wings. Females roost in summer nursery colonies, while solitary individuals roost individually in trees along forest edges, hedgerows and shrubbery borders. They migrate south in September or October, and when they return, they have one to five young early in the summer that are weaned within 6 weeks of birth. *L. cinereus* is the largest bat in Pennsylvania, but is uncommon in the state. They roost in trees (usually conifers) in forests, along forest edges and near farmland. They migrate to the coast for the winter, and return in the summer to forage and bear 1-4 pups in May-June; these juveniles learn to fly in approximately 33 days. *P. subflavus* are found throughout Pennsylvania (except in the southeast) and inhabit open woods near water, rocks, buildings or caves. They enter hibernation in September and are one of the last bats to emerge in late April and May. Two pups are often born to each mother in June or July and are weaned by their fourth week. *L. noctivagans* are solitary bats that inhabit wooded areas near lakes and streams and that roost in dense foliage and hollow trees. They migrate south for the winter to hibernate, and often go farther north or to the cooler tops of mountains to breed. They usually have one or two young born in June or July (Fergus, 2010).

Echolocation and Communication:

All members of the Vespertilionidae family are insectivorous, and they all use echolocation to forage and capture prey. Although every species of bat has ocular vision, which they can use for long-range orientation, their eyes lack cones and a tapetum lucidum (Fenton, 1983). This renders most bats' eyes to be too weak for nocturnal navigation and foraging. As a result, most species rely primarily on echolocation to perform these tasks. Echolocation is a form of seeing with sound (Yalden & Morris, 1975), in which a bat transforms acoustic information from biosonar echoes into spatial representations of objects (Simmons et al., 2004); in other words, the bat emits sound waves and then orients itself based off the echoes created by each sound wave bouncing off a surface or various surfaces.

As with all sound waves, those emitted by bats can vary in frequency, wavelength, tone and amplitude. The frequencies of bats vary between species and can even vary in a single call. Most bats use frequencies in the ultrasonic range (20 kHz and higher), which are inaudible to humans, with the most common frequencies being between 20 kHz and 130 kHz. However, some bats can reach frequencies as low as 12 kHz and as high as 160+ kHz (Yalden & Morris, 1975). Because frequency is inversely related to wavelength, the high-frequency calls of bats have very short wavelengths (Yalden & Morris, 1975). These shorter wavelengths provide a better resolution of target detail allowing the bat to receive a more detailed picture of its target (Fenton, 1983). Additionally, target strength, or the acoustic energy reflected from a target, is theoretically greatest when incident wavelengths of calls are near the same size or smaller than the target (Jones and Rydell, 2003); otherwise, if the object is smaller than the wavelength, the sound will be scattered instead of reflected (Yalden & Morris, 1975). These attributes of bat calls are what allow insectivorous bats to prey on such small targets as dipterans.

Although the high-frequency, short-wavelength calls are the reason for bats' success as nocturnal insectivores, these characteristics of their calls provide various limitations for the bats. The higher-frequency, shorter-wavelength sounds tend to be more directional, requiring the bats to turn their heads frequently to survey a broad range of area. More importantly, these types of sounds are more subject to environmental attenuation (Fenton, 1982), which causes the ultrasonic calls to have shorter effective ranges (due to already weak echoes only being effectively heard with certain intensity and distance). This forces the bats to need to be close to small targets in order to detect them. The range is typically 1-5 m for frequencies around 20 kHz and increasingly closer for higher frequencies (Jones and Rydell, 2003). The frequencies that bats use therefore have to compromise between maximizing echo strength from insects and minimizing energy loss by atmospheric attenuation ((Jones and Rydell, 2003).

The tones of a bat call can vary due to differences in frequency. As a result, calls can contain a fundamental, or base frequency, as well as one or more harmonic frequencies. Harmonics provide the bats with more detail about their surroundings (Fenton, 1982); this is because the addition of harmonic tones (or even overtones) increases the bandwidth of the calls, which can enhance target ranging and localization (Jones and Rydell, 2003) and thus increase acuity (Fenton, 1983). Some bats are able to suppress either the fundamental or one or more of the fundamental's harmonics from its calls. This provides the possibility to create varying combinations of tones and even the ability to produce a pure tone. For example, Fenton and Bell (1979) showed that *M. lucifugus* uses the fundamental and two harmonics while approaching a target, and uses just the fundamental and one harmonic right before attacking a target. Bats can also vary the amplitude, or loudness, of their calls (Yalden & Morris, 1975). Initial call intensity can greatly affect the effective range of echolocation (Fenton, 1982) and is important for indicating the position of targets for FM bats (Yalden & Morris, 1975).

Bat calls can be composed of frequency modulated (FM) or constant frequency (CF) components or a combination of both types of calls (Jones and Rydell, 2003). All members of the family Vespertilionidae use frequency modulated (FM) calls; each signal, consisting of discrete pulses of sound (Fenton, 1983), starts at one frequency and sweeps down to another. For example, a typical pulse of *E. fuscus* sweeps from 80 kHz – 30 kHz and each pulse of *M. lucifugus* sweeps from 100 kHz – 40 kHz (Fenton, 1983). By using FM calls that sweep through a range of frequencies, bats achieve some protection from errors associated with Doppler shifts; in addition, the modulation of frequencies helps to tell the bat about the texture of the target (Fenton, 1983). The echolocation signals of FM bats are produced in the larynx and emitted through the open mouth (Yalden & Morris, 1975).

Bats can alter different components of individual pulses to emphasize accuracy in location (as achieved with broadband FM signals) or accuracy in detection (best achieved with narrowband, constant-frequency signals) (Jones and Rydell, 2003). Broadband FM signals usually sweep downward through about an octave in a short time and can provide accurate measures of the range and angle of a target. These calls are well suited for prey localization, especially within vegetation. Bats that search in open areas or in edge habitats on the other hand, often add a longer narrowband component to their signal, which, by focusing the bulk of the energy in a relatively narrowband component, facilitates detection of distant objects. Narrow neuronal filters can then detect “acoustic glints” associated with modulations in the frequency and amplitude of echoes from fluttering insect wings (Jones and Rydell, 2003).

While foraging, FM bats create a series of repeating ultrasonic pulses in various phases. There are three major phases, each with an increase in repetition rate. The change in rate across the phases can go from 4-5 pulses per second to 200 pulses per second. The initial phase is the search phase, which is a period of steady low-rate pulse repetition used when searching for prey. This initial phase actually consists primarily of the bat flying in silence (Yalden & Morris, 1975), with aerial-hawking bats producing pulses at a rate of approximately one per wing beat or even as little as one per every two or three wing-beats in open habitats (Jones and Rydell, 2003). The approach (intermediate) phase occurs as the bat draws closer to a target, and the terminal phase (often referred to as the terminal or feeding “buzz” because of the sound created from the high repetition of pulses associated with the phase) occurs as the bat attempts to grab its target. Three correlated changes occur during the approach and terminal phases: the length of each pulse shortens, the interval between pulses is reduced, and the range of the frequency-drop during the pulse is lowered (Yalden & Morris, 1975). The reasoning behind these changes is described later. In addition, approach and terminal phases typically contain broadband components to optimize ranging performance (Jones and Rydell, 2003).

The key to echolocation as a form of orientation is the echo created by the sound wave of the bat’s pulses. For echolocation to work, the bat compares each outgoing call it produces with the returning echo and then determines the difference between the echo and the original sound. The difference between the two provides the bat with information regarding its surroundings and/or target (Fenton, 1983). Since sound travels in air at a constant speed of 340mm/sec, the time an echo takes to return from an object will itself indicate distance (Yalden & Morris, 1975); in other words, bats determine the range, or target distance, from an object from the delay of echoes, which is approximately 5.8 ms/m (Simmons et al., 2004). This process actually involves an overwhelming array of neural operations (Fenton, 1983).

For bats using frequency modulated calls, the intensity (loudness) of the echoes is important for indicating the position of a target (Fenton, 1982). Because echoes are usually of weak intensity, bats' hearing is very sensitive to variations in the amplitude of sound. Due to the importance of picking up the echoes of the echolocation calls, bats' brains are designed to respond more strongly to an echo than to the emitted pulse (Fenton, 1982). In addition, all vespertilionids have a fairly simple but large pinna with a small accessory lobe of skin called the tragus in the middle of the pinna. These external ear structures function to not only increase hearing efficiency, but also more importantly to determine the direction from which echoes are coming. Bats' ears are most sensitive to sounds coming from about 35° to the side and 30° below the line of flight rather than to sounds returning from straight ahead (Fenton, 1982).

In addition to target range, bats perceive target shape and texture from the delay of multiple reflections (echoes). In order to be able to use multiple reflections, a bat needs to be able to separately perceive the delays of overlapping echoes. This requires the bat to recover information about the echoes' very small delay separation and ultimately reconstruct the range profile of targets from the echo spectrum (Simmons et al., 1998). As Simmons et al. (1998) showed in an experiment with *E. fuscus*, in which the bats could perceive echoes arriving as little as 2 μs apart, the bat's two-point threshold, or the minimal spacing between two objects for which the bat could register each object individually, was 0.3 mm. This two-point resolution is approximately five times smaller than the shortest periods in the bat's sounds. Because the resolution of delay determined by their experiment seems overly sharp simply for catching insects, it is probable that the bat's biosonar images are suited to be used for a wider variety of orientation tasks such as perceiving shape and texture of surfaces or determining the locations of multiple reflecting surfaces, especially in more complicated acoustic surroundings (Simmons et al., 1998). This is especially important for when bats attempt to detect prey in complex habitats such as within foliage. Background clutter is produced by the echoes reflecting off the foliage, and this background noise often masks the echoes from insect targets making prey capture more difficult (Jones and Rydell, 2003).

In another study of *E. fuscus*, Simmons et al. (2004) found that the delay accuracy deteriorates as frequencies are removed from echoes. They found that accuracy declines consistently with the reciprocal of the bandwidth of the echo relative to its frequency, as given by the equation $Q = f_c / \Delta f$. The value of the quantity Q represents the sharpness, or quality, of the filtering imposed on echoes independent of frequency, but it is also representative of the relative width of the target impulse response in number of cycles or peaks. The importance of this value is made apparent from results of a previous experiment that showed that *E. fuscus* appears to perceive the shape of targets in terms of the distribution of reflecting points along the range axis, which is equivalent to the distribution of peaks in the target's impulse response (Simmons et al., 2004).

Bats that use FM calls keep outgoing pulses and returning echoes separate in time because they cannot detect echoes while producing echolocation calls; in other words, they are intolerant of pulse-echo overlap. This is primarily due to the contraction of two middle ear muscles, the stapedius and the tensor tympani, during call production that act to dampen the sensitivity of the ears to prevent the bats' calls from damaging them. Because the amplitude of an echo is generally much weaker than a call pulse, the echo cannot be heard until the muscles relax, which occurs approximately 5 ms after a pulse is emitted (Yalden & Morris, 1975). While foraging, the interpulse interval and pulse duration are both decreased across the three phases to avoid pulse-echo overlap as the bat moves closer to its target (Jones and Rydell, 2003). In addition, after each pulse has been emitted, the bat has to wait to process any usable echoes before emitting the next pulse. As a result, only occasional pulses with long listening periods are necessary during the search phase. As the bat draws closer to a target, it becomes more important for the bat to be constantly updated on the position of its target and therefore it sends out pulses more frequently during the approach and terminal phases. As it gets closer to its prey, however, the echoes will return more quickly, and, due to the necessity to avoid pulse-echo overlap, this necessitates shorter pulses (that, as a result, drop across a briefer span of frequencies) to enable the rapid accession of information (Fenton, 1982).

The signals bats produce may serve multiple functions; they can be used for echolocation as well as for communication, whether intentionally or unintentionally. For example, *M. lucifugus* was shown to be attracted to the calls of conspecifics from up to 50 miles away as well as to the calls of *E. fuscus*. *E. fuscus* was similarly attracted to the calls of conspecifics as well as calls of *M.* lucifugus (Altringham and Fenton, 2003). In addition, little browns have been shown to use echolocation calls of others to locate roosts, and eavesdropping also allows a territorial bat to distinguish between another bat trying to steal its food or just one passing through its territory (Fenton, 1983). These results suggest that bats can eavesdrop on other bats to locate potential foraging sites. It has also been suspected that modifications of call structure (e.g. bandwidth, duration, and interpulse interval) may enhance communication function. Many bats actually emit calls during flight that differ in structure from echolocation calls; these calls, often referred to as "social calls," may occur between echolocation calls and often consist of broadband calls with low-frequency components and multiple syllables (Altringham and Fenton, 2003).

Bats also use a variety of other calls. They use alarm calls and distress calls when in stressful situations, as shown by an experiment in which *M. lucifugus* dove at speakers projecting distress calls. Studies of *M. lucifugus* have also shown that males produce long, low-frequency vocalizations during copulation; these calls actually attract conspecifics males, which try to interrupt copulation (Altringham and Fenton, 2003). Some bats use special sounds to avoid collisions with each other while in flight (Fenton, 1983). Mother-young communication also exists among bats. For example, when searching for her pups, a female bat will produce "directive" calls consisting of intense, rapidly repeating distinctive sounds that attract young bats. In some species, such as some vespertilionids, early communication calls of young bats may be precursors to echolocation calls; call duration decreases and frequency increases as young bats develop (Altringham and Fenton, 2003).

Bioacoustics and Call Analysis

Bioacoustics is a science that combines both biology and acoustics by studying the sound production and communication of animals. Bioacoustical recorders are often used to study whales, insects, elephants, bats and most often birds and birdcalls. Because bats produce calls in the ultrasonic range, special ultrasonic recorders are necessary to monitor bats with bioacoustics. In contrast to trapping, identification and monitoring of bats from recording echolocation calls provides a noninvasive method that can be applied over a wide area with low labor costs and that can provide detailed species-specific data (Parsons and Szewczak, 2009).

After calls have been recorded with an ultrasonic recorder, they must be analyzed both spectrally and temporally. Temporal parameters include call-duration and the interpulse interval, or time between calls. This information can be measured directly from an oscillogram, which is a graphical representation of the sound wave that has been recorded by the microphone. The frequency content of the calls is often analyzed using spectrograms, which graphically display signal frequency, duration, and amplitude. Because the echolocation calls generally varies in frequency, duration, repetition-rate, frequency-time structure, and amplitude across species, these parameters can be used to distinguish individual species from their calls. Generally, modern software programs such as SCAN'R (Binary Acoustic Technology LLC) have their own call libraries and are able to determine both the number of calls recorded and the species making the calls (Parsons and Szewczak, 2009).

Although bat call recording is used frequently to monitor bats (Fukui et al., 2006; Ford et al., 2006; Gehrt and Chelsvig, 2004; Gehrt and Chelsvig, 2003; Barclay 1991; Aldridge and Rautenbach, 1987), there are issues that arise with this method of monitoring. Monitoring bat populations is challenging because bats are cryptic, use large geographic areas in three-dimensional space, and have the potential to disperse. Bat populations are rarely static, and thus results of monitoring efforts can be extremely sensitive to spatial and temporal factors. Therefore, monitoring efforts need to be conducted for sufficient periods of time and over large geographic expanses. Although restricting efforts to areas where bats are expected to commute, forage, or drink is a common practice because it often maximizes detection rates, data collected in this manner typically are not representative of populations as a whole. The data are biased toward those species or individuals that use the habitat features in the particular areas selected and biased against those that do not (Parsons and Szewczak, 2009). Additionally, although most bioacoustical monitoring takes place over bodies of water shortly after dusk, these conditions are typically poor for recording. Just after dusk, the air cools over a still-warm landscape resulting in thermal convection currents that can distort sound. Bodies of water, which retain a temperature contrast with the air, are also potential sources of such refractive distortion (Parsons and Szewczak, 2009).

Habitat:

In temperate regions, habitats associated with woodland, water, and woodland/grassland edges are used by the broadest range of bats. Old-growth forests seem to be of greater importance for both levels of bat activity and associations of individual species of bats than logged or younger stands. Bat activity can also be greater in less cluttered areas due to the accessibility created for open-air or gap-using species. In fact, when short-term changes occur in a forest due to newly cut areas or the installation of access roads, bat activity can actually increase due to the increase in accessibility to edge feeders and commuting bats to the unaffected insect availability of the affected areas. Studies have shown that deforestation can significantly change the composition of bats in an affected area by causing a decrease of rarer forest-dependent species while allowing the more common edge-inhabiting species to thrive (Racey and Entwistle, 2003).

In forested areas, the distribution of bats is influenced by a complex interplay of habitat factors; these factors range from the individual tree and stand-level to the landscape level. Prey resources, temporal factors, climate, and the autoecology of individual bat species are all factors. The location of a bat at any instance can depend on a multitude of factors as well, such as time, temperature, humidity, precipitation, barometric pressure, wind speed, ambient light intensity, and the availability and abundance of prey insects. On a large landscape scale, bat presence is often related to the abundance of day roosts, the proximity of foraging habitat to the day roosts, the quality and density of available foraging areas, and the amount and type of disturbances that affect that landscape. On a smaller habitat scale, activity is often related to structural characteristics of the forest, proximity of riparian habitat, and characteristics of the bat species themselves (e.g. echolocation characteristics, wing morphology, and prey preference) (Ford et al., 2006).

Ford et al. (2006) found that the presence of bats was generally linked to microhabitat or stand-level structural conditions as well as to the proximity to riparian habitat. They also found that the distribution of bats within pine stands, hardwood stands, and open areas were positively related to the proximity to water and the presence of canopy gaps.

Although cluttered forest areas often contain higher densities of insects, reduced clutter can result in relatively greater availability of insects for bats even with less insect abundance. This is because the ease of maneuverability and movement while foraging in a more open area overcomes the benefits of greater prey abundance in more cluttered areas (Ford et al., 2006). This is probably one of the reasons why bats are linked to riparian habitats. Streamside riparian zones are especially important for many insectivorous species because they serve several ecological functions for bats. First, they provide favorable structural habitat; the wider gaps created by streams in forests can facilitate travel and foraging, and they also provide structural edges that are preferred habitat for many species. Secondly, riparian vegetation provides shelter from wind and predators. For example, tree cover suppresses light intensity to a degree, which allows the bats to emerge earlier in the evening with a lower risk of predation. Most importantly, streams and riparian habitats provide an abundant source of prey insects for bats (Fukui et al., 2006). Although bat activity differs between pool and riffle lengths, bats frequently use the space above all lengths of streams, as well as above ponds and riparian vegetation to forage and feed on emerging aquatic insects (Fukui et al., 2006).

Foraging:

Bat foraging is affected by a number of factors. Temperature conditions directly affect the seasonal changes in bat activity levels. Emergence from the winter roost occurs only when ambient temperatures exceed 4°C (Erkert, 1982), and most bats will not usually leave their day roost to forage at night unless the temperature is above 10°C. The temperature limitations are most likely due to the fact that the insects that the bats prey upon become common only when ambient temperatures exceed 10°C (Fenton, 1983) and that overall insect abundance is lower with colder temperatures (Anthony & Kunz, 1977). Kunz (1974) found that another Myotis species, *M. velifer*, foraged in open areas adjacent to wooded vegetation on warm nights, but restricted their foraging to densely vegetated areas on cool nights. However, Kunz found there was no evidence that variation in ambient temperature had any influence on nightly emergence during the warm months of summer.

Precipitation has also been shown to affect bat foraging. Light precipitation, as well as light wind, have little or no effect on emergence or foraging (Kunz, 1974), whereas heavy precipitation and/or strong winds can restrict foraging activity by reducing flight time, causing later emergence or by causing the bats to not emerge at all (Erkert, 1982). This reduction or lack of foraging is possibly due to the ineffectiveness of high frequency bat calls in heavy rain due to both the high atmospheric attenuation from increased humidity and the immense background noise produced by extra echoes off raindrops. Turbulence from wind can also generate refractive distortion of ultrasound (Parsons and Szewczak, 2009). Additionally, wet fur and wings increase the evaporative cooling that the bats are subjected to, thus affecting their temperature regulation (Fenton, 1983). Lactating females, which have been shown to forage even during thunderstorms, seem to be the exception to the effects of precipitation on foraging activity (Fenton, 1983).

Emergence, and therefore foraging, is affected by light levels (illumination intensities) as well. In fact, results of many studies suggest that light is the most important factor influencing evening emergence (Kunz, 1974). Cloud-cover, which affects both the scattering and blocking of light, plays a major role in the effect of light as a factor that affects foraging activity. During partly cloudy conditions, the sky remains bright longer during twilight periods than under clear conditions (due to reflection of light on the clouds from the setting sun), which causes the bats to emerge later in the night. During completely cloudy evenings, bats are able to emerge earlier to forage because the clouds block the light from the setting sun earlier in the evening (Kunz, 1974). Cloudy conditions throughout the night also reduce moonlight. Moonlight has been shown to depress the activity of bats, although the reason is unknown (Fenton, 1983). The sequence of when the first bats leave their roosts and the last bats return is usually consistent. Erkert (1982) suggests that physiologically, light does not control the emergence /return activity rhythm directly, but instead its primary role is synchronization of an endogenous timing system for circadian rhythm. The timing of return flights to the day roosts is actually influenced primarily by foraging success and weather conditions, and it is dependent on the amount of time that the bats are removed from foraging and instead occupying night roosts (Erkert, 1982).

Insectivorous bat foraging also differs with time of the night. Levels of activity change throughout the night, often resulting in a bimodal pattern of foraging. Although some bats have been found to be active throughout the night by monitoring echolocation calls, generally most bats experience two major peaks of activity each night (Fenton, 1983). Most species of bats do not begin to forage until the late evening twilight (Erkert, 1982). The first approximate two hours of foraging (lasting from around sunset until 2200 or 2400 h local time) make up the first major foraging peak (Anthony and Kunz 1977). This peak in activity is actually much greater than the second peak of the two major peaks, and it is during these first couple of hours that approximately 80% of the bats' daily food consumption is accounted for (Kunz, 1974). Anthony and Kunz (1977) found that within the first 20 minutes of the first peak of activity, pregnant females consume approximately 20% of their total nightly intake, while lactating females consume approximately 30% and juvenile bats consume approximately 40% of their total nightly meal. Overall food intake is generally much less after midnight compared to the first foraging period. The second peak is less active than the initial foraging bout, and it usually lasts for approximately two to three hours before sunrise. Juvenile bats, which overall consume less insects than adult bats, initially use a unimodal pattern of foraging activity after first becoming volant. This single peak occurs during the first major peak of adult activity. The juveniles switch to a bimodal pattern once they develop an adult-like flight behavior (Kunz, 1974).

Anthony and Kunz (1977) showed in their study of *M. lucifugus* that over 60% of the bats' total nightly intake occurred before midnight. The high level of consumption they found was correlated with the time that insect availability was the highest, as indicated by suction-trap sampling. Activity decreased sharply after midnight, with an occasional secondary peak of activity before sunrise. Similarly, Barclay (1991) showed through sticky-trap sampling that the early period before midnight had significantly higher total insect mass than either the middle or late periods of the night – the high abundance of prey drops by almost an order of a magnitude a few hours after sunset. These data, as well as the results of other studies, suggest that the predominantly bimodal activity pattern of insectivorous bats is a response to the times of prey availability (Erkert, 1982).

Prey availability varies not only throughout a single night but also throughout the year. Barclay (1991) showed through sticky-trap capture that in addition to temporal effects, there are also seasonal and habitat-related effects on the mass of insects available. Bats in temperate zones hibernate to conserve energy during the winter because of the scarce level of prey insects (Erkert, 1982), which do not begin to emerge until ambient temperatures exceed 10^{O}C in the spring (Fenton, 1983). Although overall resource densities are low in spring (Kunz, 1974), Fukui et al. (2006) found that adult aquatic insect biomass was the highest in the spring and decreased gradually throughout the summer; in contrast, terrestrial insects nearby the riparian study area were lowest in the spring and gradually increased during the summer (due to an increase in terrestrial plant productivity). Additionally, Anthony and Kunz (1977) showed that aquatic insects numbers, quantified from suction-trap capturing, varied due to temperature in June, reached a peak in early-mid July, and declined during August.

Seasonal effects, as well as sex and age differences, are also factors that influence the activity level of foraging bats. Results from studies of *M. lucifugus* (Anthony & Kunz, 1977) and *M. velifer* (Kunz, 1974) showed consistent results in the seasonal feeding habits of temperate insectivorous bats. The feeding habits change seasonally in response to not only insect abundance, but more importantly in response to changing energy demands, which have evolved themselves in response to the seasonal availability of resources. Maximum daily food intake for adult males occurs in early summer, corresponding to the period of molt and the initiation of spermatogenesis. Food consumption increases in females throughout pregnancy during the spring and early summer, and continues to increase even after parturition in order to meet the demands of milk production. Consumption levels reach a maximum level for lactating females in midsummer during the latter stages of pre-weaning development when demand for milk is greatest. The feeding efficiency of juvenile bats is relatively low compared to adult levels until mid to late summer (July-August) when they develop adult patterns of feeding and are able to forage the entire night. Juveniles must increase their consumption in order to build fat reserves for the winter. Overall, food consumption in bats decreases in late summer and in autumn.

Prey are often captured by insectivorous bats midflight. Small prey are caught directly by the mouth (Fenton), while some prey are brushed to the mouth using the wing. Sometimes food is transferred to the tail, which is formed into a pouch, where the bat bends its mouth down to eat also while flying. If the prey insect is too large or unwieldy for these eating methods, they are carried back to a night perch and eaten there (Yalden & Morris, 1975). Although different bat species have some difference in the size of insects that they frequently eat, the sizes taken by many species overlap with other species (Yalden & Morris, 1975). Brosset distinguished between long-term and short-term bats: some species react to prey at relatively farther distances and only make one attempt to catch a prey insect per pass through a foraging area, while other species operate at short ranges, usually less than 1m (e.g. *M. lucifugus*), and make several attempts to capture prey on any pass through a feeding area (Fenton, 1982).

Insects eaten by bats must be small enough to be caught by the bat, but large enough to be energetically efficient to prey upon (Whitaker, 2004). Often, insects, especially Dipterans, less than 2 mm in length are too small to be detected by many bats, due to call wavelength limitations. *M. lucifugus* for example, have been found to prey on insects ranging from 3-10 mm in body length. The upper limit of prey size is often determined by the increase in time and effort in capturing and handling larger insects, which eventually becomes inefficient (Anthony & Kunz, 1977).

Overall, most insectivorous bat species (e.g. *M. lucifugus and E. fuscus*) are opportunists. The bats eat a wide range of insects, often eating insects that are the most abundant and diverse and that are within the appropriate prey size range. Barclay and Brigham showed that movement is actually a major cue that a bat will use to decide to pursue an object as a prey item (Jones and Rydell, 2003). Although bats are opportunists, they can be considered selective in diet because they often exploit local insect faunas that are overly abundant (Anthony & Kunz, 1977). In areas where single or minimal species populations predominate and where interspecific competition is minimal, prey items are able to be selected for their abundance and relative energy value. In this case, size may only be a limiting factor with respect to upper and lower size limits and palatability (Kunz, 1974). Size itself may cause selection, in that larger insects, such as Coleopterans, are easier for bats to spot and therefore pursue than smaller insects. Size may also determine the range of insects that specific bat species specialize in eating.

Additionally, Anthony and Kunz (1977) found that selectivity can vary among species themselves. They showed that non-pregnant female *M. lucifugus* are selective in their diet during periods of high insect abundance, but pregnant females feed more randomly, most likely due to the need to consume larger amounts of food for their young. They also showed that juveniles fed non-selectively. Whitaker (2004) found that level of selectivity also varies with season. Selectivity is minimal or absent early and late in the season and during times of low insect abundance because the bats have little selection from which to choose. In contrast, when insect abundance and diversity is greater, especially during midsummer, selection of specific species increases, most likely due to size and palatability differences.

Selectivity itself can change throughout the season as well. In a study using insect-proof covers on a 2-5m wide stream, Fukui et al. (2006) found that bat foraging was heavily dependent on aquatic insects early in the season. However, the importance of aquatic insects as a prey source decreased throughout the season when abundance decreased and the importance of terrestrial insects increased as terrestrial abundance increased over time.

Many orders of insects are eaten by bats, but nocturnal swarms of Diptera, Hymenoptera, Isoptera, Ephemeroptera, Trichoptera, and some Neuroptera are often exploited by bats; these are insects that typically form crepuscular or nocturnal swarms over water. Bats tend to exploit swarming insects because they are both visually and acoustically conspicuous. They often hunt in aquatic and riparian habitats extensively because the swarms of insects over water require less searching and are more conspicuous than prey in terrestrial habitats. Exploitation of aquatic swarms is particularly important for bats at high altitudes in Europe and North America because these areas have habitats with numerous lakes and rivers (Jones and Rydell, 2003).

More Information about Common Species Expected to be Monitored During the Study:

The most common species of bat in Pennsylvania is *M. lucifugus*. Characteristics of the echolocation call and wing morphology of *M. lucifugus* both indicate that little browns are more adapted to feeding in a less cluttered environment (Aldridge and Rautenbach, 1987), such as over water (Brack, 2009). Results from many studies support their abundance and frequency over streams (Anthony & Kunz, 1977; Barclay, 1991; Brack, 2009). They have been found to spend most of their time foraging within 50 cm of the surface of the water (especially calm water), and have been observed frequently touching the water as if taking prey from it (Barclay, 1991). *M. lucifugus*, like most Myotis species, tend to forage in small local patrols by flying around a small area repeatedly, instead of traveling far and frequently throughout the night (Yalden & Morris, 1975). Little browns feed frequently on Diptera, Coleoptera, Trichoptera, Lepidoptera, Ephemeroptera, and Neuroptera (Anthony & Kunz, 1977), and they have been found to catch over 1 g (500+) insects per hour (Yalden & Morris, 1975).

Big brown bats, *E. fuscus* are also common in Pennsylvania, and their abundance, like the little brown bats, has been found to be positively correlated to less distance from water (Ford et al., 2006). Also, although *E. fuscus* is often considered a generalist in its type of habitat use, Brack (2009) captured this species at a disproportionately high rate at riparian sites, compared to other habitats. Due to their larger size, big brown bats often eat larger, often heavily-chitinized, insects, such as beetles (Coleoptera). Additionally, they have been found to prey frequently upon Hymenopterans, Dipterans, Plecopterans, Ephemeropterans, Hemipterans, Trichopterans, Neuropterans, Mecopterans, and Orthopterans (Yalden & Morris, 1975).

Streams and Water Quality

Aquatic macroinvertebrates are important components in stream assessment. Due to varying tolerances of water quality (e.g. chemical characteristics, pH, oxygen levels, temperature, physical characteristics, etc.) amongst aquatic macroinvertebrates, the presence of specific organisms can be used as an indication of stream quality. Streams affected by disturbances such as livestock grazing, incompletely treated sewage and suburban development have been found to sustain fewer than 20 taxa of macroinvertebrates compared to the 24-40 taxa of a typical pristine stream. Streams affected by abandoned mine drainage (AMD) show an even greater loss of diversity, often losing the ability to sustain macroinvertebrate life at all (Walter et al., 2010).

AMD is the result of a chemical reaction of surface water (e.g., rainwater, snowmelt, pond water) and shallow subsurface water with rocks that contain sulfur-bearing minerals, resulting in the formation of sulfuric acid. In the case of pyrite (FeS_2), oxidation of the ferrous iron into ferric ions occurs, which react with the pyrite, thus further lowering pH values. The acidic AMD can leach out heavy metals from rocks that are exposed to the acid, resulting in highly toxic water that can mix and contaminate groundwater, surface water (i.e. streams) and soil. The acidic AMD can become neutralized if it comes into contact with carbonates such as limestone, which can reduce, but not necessarily eliminate, the detrimental effect the AMD has on the soil or water that it enters. However, if the pH is increased to levels of 6.0 or higher, the alkaline mine drainage that results can have damaging effects (EPA, 1994).

As described in Walter et al. (2010), abandoned mine drainage usually involves concomitant changes in pH, dissolved and precipitated metals and sedimentation. Fluctuations in pH levels and or stream chemistry have been found to limit macroinvertebrate diversity and abundance due to changing the levels to those outside the organisms' optimal ranges. Additionally, some of the dissolved metals can be toxic, e.g., arsenic, copper and zinc, and will reduce the viability of stream organisms. Metal hydroxides, including iron and aluminum, are common components of coal mining discharges in the US and around the world. Certain metal hydroxide particulates have impacts similar to those of common sediments from agriculture, development, logging and sewage, and therefore have similar physical problems for stream organisms. For example, fine particles can coat surfaces of stream invertebrates and reduce surface oxygen exchange, block attached algae, slow leaf decomposition, reduce protective hiding places by filling up gaps between rocks and form unstable surfaces on rocks and stream debris. Residual metal hydroxide deposits have been found to further impair ecosystem functions by leading to reduced litter processing, consumption of metal contaminated biofilm by organisms within the stream and direct contact with toxic metals in hyporheic retreats. Additionally, lower macroinvertebrate diversities and densities within a stream result in lower adult aquatic insects around the stream. Lower aquatic insect levels will reduce the availability of food for riparian foragers such as birds and insectivorous bats.

Multiple solutions have been devised to reduce the disturbances of streams due to AMD. Lime or other alkaline substances can be periodically added to streams to neutralize the pH of the affected streams. The creation and utilization of wetlands can also improve stream conditions. As AMD-effected water flows through the wetland, the concentration of contaminants is reduced through two major processes. Contaminants are removed primarily through chemical (e.g. oxidation and precipitation of metals) and biological (e.g. collection and filtering of metals by cattails) processes; the contaminants can also be diluted by inputs of uncontaminated water (Hedin and Nairn, 1993).

Project Rationale

Conservation of bat populations is important in order to maintain biodiversity and, on a practical level, control insect populations. First, stream quality is important for bat conservation because waterways such as streams are often used by insectivorous bats for foraging. The results of this project will help to determine if decreased water quality, specifically water impacted from abandoned mine drainage, will decrease macroinvertebrate and aquatic insect abundances and therefore result in a decrease in bat foraging of affected areas. Secondly, monitoring bats in the beginning and end of the active season is important to understand population levels and potential future impacts of climate change on animal phenology.

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APPENDIX D

Poster:

This poster was presented at the National Conference on Undergraduate /research (NCUR) on April 2, 2011 in Ithaca, NY and at the 8th Annual Academic Conference at Saint Vincent College on April 13, 2011.